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Common variants in 22 loci are associated with QRS duration and cardiac ventricular conduction

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Abstract

QRS interval on the electrocardiogram reflects ventricular depolarization and conduction time, and is a risk factor for mortality, sudden death, and heart failure. We performed a genome-wide association meta-analysis in 40,407 European-descent individuals from 14 studies, with further genotyping in 7170 additional Europeans, and identified 22 loci associated with QRS duration ($P < 5 \times 10^{-8}$). These loci map in or near genes in pathways with established roles in ventricular conduction such as sodium channels, transcription factors, and calcium-handling proteins, but also point to novel biologic processes, such as kinase inhibitors and genes related to tumorigenesis. We demonstrate that *SCN10A*, a gene at our most significant locus, is expressed in the mouse ventricular conduction system, and treatment with a selective *SCN10A* blocker prolongs QRS duration. These findings extend our current knowledge of ventricular depolarization and conduction.

Search Terms

QRS interval; ECG; quantitative trait; genome-wide association study

The electrocardiographic QRS interval reflects ventricular depolarization and its duration is a function of electrophysiological properties within the His-Purkinje system and the ventricular myocardium. A diseased ventricular conduction system can lead to life-threatening bradyarrhythmias, such as heart block, and tachyarrhythmias, such as ventricular fibrillation. Longer QRS duration is a predictor of mortality and sudden death in the general population and in cohorts with hypertension and coronary artery disease.1–3 In a population-based study, prolonged baseline QRS was associated with incident heart failure.4

Twin and family studies suggest a genetic contribution to QRS duration, with heritability estimates of up to 40%.5, 6 Prior candidate gene and smaller genome-wide studies identified a limited number of loci associated with QRS duration, supporting the hypothesis of the contribution of common genetic variation to QRS duration.7–9 To identify additional loci and highlight physiologic processes associated with ventricular conduction, we performed a meta-analysis of 14 genome-wide association studies (GWAS) of QRS duration in a total of 40,407 individuals of European descent, where we adjusted the analyses for age, sex, height, and body mass index after appropriate sample exclusions (**Methods**). After an initial discovery phase, we further genotyped selected variants representing nine loci with *P*-values ranging from 1×10^{-6} to 5×10^{-9} in an additional cohort of 7170 European individuals.

Results

Meta-analysis of genome-wide association results

We conducted meta-analyses for approximately 2.5 million single nucleotide polymorphisms (SNPs) in 40,407 individuals of European ancestry from 14 GWAS (Supplementary Tables 1a and 1b). Overall, 612 variants in 20 loci exceeded our genome-wide significance *P*-value threshold of 5×10^{-8} after adjusting for modest genomic inflation ($\lambda_{GC} = 1.059$) (Figure 1 and Supplementary Figure 1). The loci associated with QRS interval duration are detailed in Table 1 and Supplementary Figure 2, with the index SNP (representing the most significant association) labeled for each independent signal.

Across the genome, the most significant association for QRS interval duration (locus 1) was on chromosome 3p22 (Figure 2a), where we identified six potentially independent association signals based on the linkage disequilibrium (LD) patterns in HapMap-CEU (pairwise r^2 among index SNPs < 0.05). In conditional analyses where all six SNPs were included in the same regression model, there was compelling evidence that at least four SNPs from this region were independently associated with QRS duration (Table 1). Two of these associations were in or near *SCN10A,* a voltage-gated sodium channel gene. Variation at this locus was recently associated with QRS duration in two GWAS. The top SNP identified in those two studies, rs6795970, was in strong LD with our top signal, rs6801975 (r^2=0.93).8, 9 Two additional signals were identified in *SCN5A*, a sodium channel gene adjacent to *SCN10A* (Table 1).

The second most significant locus (locus 2) was on chromosome 6p21 near *CDKN1A*, a cyclin dependent kinase inhibitor. The *CDKN1A* locus was recently associated with QRS interval duration in an Icelandic population.9 The index SNP in the prior report, rs1321311, was in strong LD with our top signal, rs9470361 (r^2=0.88). Another cyclin dependent kinase inhibitor (*CDKN2C)* was located in locus 15, which encompasses several other genes including *C1orf185, RNF11,* and *FAF1.*

Locus 3 on chromosome 6q22 contains the *PLN/SLC35F1/C6orf204/BRD7P3* cluster of genes. *PLN* encodes phospholamban, a key regulator of sarcoplasmic reticulum calcium reuptake. Significant associations were found in several other regions harboring calcium-

handling genes, including locus 12 (*STRN/HEATR5B*), locus 16 (*PRKCA*), and locus 18 (*CASQ2*).

Locus 4 mapped to an intronic SNP in *NFIA*, a transcription factor. Several other significant loci also mapped in or near transcription factors including locus 5 (*HAND1*), locus 6 (*TBX20*), locus 8 (*TBX5*), locus 9 (*TBX3*), and locus 19 (*KLF12*). Common variation in *TBX5* was recently associated with QRS duration.9 The index signal in the prior report, rs3825214, was in moderate LD with our top signal, rs883079 (r^2=0.67).

Additional regions identified include locus 7 (*SIPA1L1*), locus 10 (*VTI1A*), locus 11 (*SETBP1)*, locus 13 (*TKT*/*CACNA1D/PRKCD*), locus 14 (*CRIM1*), locus 17 (nearest gene, *IGFBP3*, is 660kb away), and locus 20 (*LRIG1*).

Collectively, the identified index SNPs across these 20 loci explained approximately 5.7% (±2.3%) of the observed variance in QRS duration, consistent with a polygenic model in which each of the discovered variants exerts only a modest effect on QRS interval. None of these index SNPs showed a significant interaction with sex or age after Bonferroni correction (Supplementary Table 2). We observed moderate levels of heterogeneity of the effect ($25 < I^2 < 75$) for several index SNPs (Table 1). However, only *HAND1*/*SAP30L* showed significant evidence of heterogeneity using Cochran's Q test corrected for 23 independent genome-wide variants (Cochran's $P = 0.005$).

Extension of findings in an additional 7170 individuals

Based on the discovery meta-analysis, we selected the index SNPs at four loci (loci 15, 17, 19, and 20) with *P*-values ranging between 5×10^{-8} and 5×10^{-9} and from all five loci with *P*-values ranging from 1×10^{-6} to 5×10^{-8} (**Methods**) for genotyping in an additional 7170 European individuals in order to boost power. In a joint analysis combining all 47,577 individuals, the significance for the four loci with *P*-values between 5×10^{-8} and 5×10^{-9} increased, indicating these represent true positive associations (Table 1). The joint analysis also provided further evidence for two other loci (locus 21 near *DKK1*, and locus 22 tagged by an intronic SNP in *GOSR2*) that reached genome-wide significance, bringing the total number of significant loci to 22 with 25 independently associated index SNPs (Table 1). The index SNP (rs1733724) in *DKK1* was previously associated with QRS duration in an Icelandic population.9

Association with conduction defect

Based on this series of QRS associations, we sought to test the hypothesis that QRS prolonging alleles, on average, increase risk of ventricular conduction defects. To address this question, we calculated a risk score in each individual by adding up the number of QRS prolonging alleles identified in this study, weighted by the observed effect sizes (β-estimates) from the final meta-analysis. In an independent set of 522 individuals from the ARIC and RS studies with bundle branch block or nonspecific prolongation of QRS interval (QRS>120 ms) compared with those with normal conduction ($N = 12,804$), each additional copy of a QRS prolonging allele was associated with a 8% increase in risk of ventricular conduction defect ($P = 0.004$). This result was largely driven by those with non-specific intraventricular conduction defects as opposed to those with left or right bundle branch block (Supplementary Tables 3a and 3b). Similar results were observed using an unweighted genotype risk score.

Putative functional variants

Of 612 genome-wide significant SNPs, one in *SCN5A* (rs1805124, H558R, $P = 2.4 \times 10^{-18}$), two in *SCN10A* (rs12632942, L1092P, $P = 5.1 \times 10^{11}$, and rs6795970, A1073V, $P =$

5×10^{-27}), one in C6orf204 near *PLN* (rs3734381, S137G, P=1.1×10^{-10}), and one in *CASQ2* (index SNP rs4074536, T66A, $P = 2.4\times10^{-8}$) were nonsynonymous (Figure 2 and Supplementary Figure 2). The PolyPhen-2 program predicts all five of these variants to be benign, which is consistent with small-effect associations: each copy of the minor allele was associated with cross-sectional differences in QRS duration of less than 1ms.

The 25 index SNPs (from Table 1) were subsequently tested for association with gene *cis*-expression levels in 1,240 PAXgene whole blood samples10. Four *cis*-eQTLs were detected after stringent Bonferroni correction (Supplementary Figure 3). The most striking eQTLs were observed for probes in exonic regions of *TKT* (rs4687718, $P = 5.87\times10^{-70}$) and *CDKN1A* (rs9470361, $P = 1.41\times10^{-10}$) and an intronic probe for *C6orf204* near *PLN* (rs11153730, $P = 1.54\times10^{-10}$). We additionally assessed *cis*-regulation for all HapMap SNPs for these three loci (± 250kb around the SNPs). The top eSNP for *TKT* (rs9821134) and *C6orf204* (rs11970286) were in moderate to high LD ($r^2 = 0.47$ and 0.91, respectively) with the top QRS signals at these loci. However, the top eSNP for *CDKN1A*, rs735013, was only weakly correlated with the QRS index SNP rs9470361 ($r^2 = 0.089$). In conditional analysis that included both *CDKN1A* locus SNPs in the regression model, both rs735013 and rs9470361 remained independently associated with expression levels ($P = 1.7 \times 10^{-9}$ and 2.3×10^{-5}, respectively). Additionally, rs735013 itself was marginally associated with QRS duration (coded allele frequency = 0.39; β = 0.33 ms (±0.07); $P = 2.4 \times 10–6$). Whether these associations in whole blood samples will be similar to associations in cardiac myocytes and conduction tissue deserves further investigation.

Pleiotropic effects of variants associated with QRS duration and other ECG measurements

To explore the shared genetic underpinnings between atrial and ventricular depolarization and conduction (as measured by PR and QRS intervals) as well as ventricular depolarization and repolarization (QRS and QT intervals), we examined the effects of published PR and QT SNPs with respect to QRS interval. Several QRS loci were previously associated with PR or QT intervals, including *PLN*, *TBX5/3*, and *SCN5A/10A*, the last of which is associated with all three traits (Supplementary Table 4a). We also tested nine PR SNPs and 16 QT SNPs for their effect on QRS duration (Supplementary Table 4b).11−13 Our results suggest roles for *CAV1/2* (rs3807989, $P = 5.8 \times 10^{-6}$) and *NOS1AP* (rs12143842, $P = 1.3 \times 10^{-4}$) in QRS duration. Indeed *CAV1/2* was recently associated with QRS interval.9

QRS duration is positively correlated with both PR interval (r = 0.09) and QT interval (r = 0.44).9 To test if these relationships are also observed genetically, we compared the directionality of the association of SNPs at the published PR and QT loci with those for QRS duration. Generally, the effects of SNPs on PR interval were positively correlated with their effects on QRS duration (r = 0.53). With the exception of *TBX3*, the loci influencing both PR and QRS (*SCN5A, SCN10A, TBX5,* and *CAV1/2*), do so in a concordant fashion (i.e. variants that prolong PR also prolong QRS duration) (Figure 3 and Supplementary Tables 4a and 4b). By contrast, while QT and QRS are positively correlated at the population level, the effects of SNPs on QT interval were marginally negatively correlated with their effects on QRS (r = −0.08). Of the index SNPs at the four loci significantly associated with both QT and QRS (*SCN5A*/*SCN10A*, *PRKCA, NOS1AP, PLN*), only the *PLN* locus SNPs showed effects in the same direction (Figure 3 and Supplementary Tables 4a and 4b).

Bioinformatic network analysis of QRS-associated loci

To examine the relationships between genetic loci associated with QRS duration, we developed an *in silico* relational network linking the loci based on published direct gene product interactions obtained from curated databases (Supplementary Figure 4).14 Most loci

meeting genome-wide significance mapped to this network after a minimum number of "linker" nodes were incorporated to create a spanning network. This analysis provides a graphical overview of the interconnections among QRS-associated genetic loci and highlights both known and putative molecular mechanisms regulating ventricular conduction (see **Discussion**). Several of the "linker" nodes incorporated in the network, such as calmodulin, connexin 43 (*GJA1*), *NEDD4*, *KCNMA1*, and *RYR2* are known modulators of cardiac electrical activity. Functional enrichment analysis of the QRS-associated network nodes (loci with $P<5\times10^{-8}$) using two independent software tools revealed that programs involved in heart development were highly over-represented (*P*-value range: 5.8×10^{-6} – 9.6×10^{-5}).15, 16

Functional effects of the *SCN10A* locus in a mouse model of cardiac conduction

We undertook functional studies to determine whether our most significant locus was associated with ventricular conduction in mice. Transcriptional profiling suggests that *Scn10a*/$Na_v1.8$ mRNA is expressed in ventricular myocardium and at higher levels in the specialized conduction system.17 These data were confirmed and extended by qPCR (Figure 4a), demonstrating a 25.7 ± 1.1 fold enrichment of *Scn10a*/$Na_v1.8$ in Purkinje cells compared to working ventricular myocytes (n=3 for each cell type; p=0.002).

Telemetric electrocardiographic recordings (lead II position) were obtained in conscious mice treated with A-803467, a potent *Scn10a*/$Na_v1.8$ antagonist, which blocks $Na_v1.8$ 100 times more potently than $Na_v1.5$ with the doses used.18 These studies demonstrated a significant increase in QRS duration (11.6 ± 2.6 ms to 14.5 ± .54 ms; n = 7; P<0.001), whereas vehicle alone was without effect (11.4 ± .29 ms to 11.9 ± .42 ms; n = 7; P=NS). PR interval was also increased in drug-treated mice, from 31.4 ± .98 ms to 42.5 ± 3.3 ms; n=7; P< 0.01), whereas vehicle alone resulted in no significant change (32.6 ± 1.0 ms to 33.4 ± . 69 ms; n=7; P=NS) (Figure 4b). To further delineate the site of ventricular conduction slowing, we performed intra-cardiac recordings from mice treated with A-803467. These studies confirmed the significant increase in QRS duration (from 12.26 ± 0.62 ms to 14.56 ± 0.58 ms; n=7; *P* =0.015), whereas vehicle alone was without significant effect (12.39 ± 0.52 ms to 13.65 ± 0.97 ms; n = 5, *P* = NS). A-803467 treatment resulted in a 35.7% ± 1.2% increase in HV interval (from 9.33 ± 0.74 ms to 12.67 ± 1.06 ms; *P* = .009), whereas vehicle alone was without significant effect (10.67 ± .83 ms to 11.17 ± 1.10 ms; *P* = NS) (Figure 4c). Taken together, these data indicate that the QRS prolongation may primarily reflect conduction slowing in the specialized ventricular conduction system.

Discussion

Our meta-analysis of 14 genome-wide association studies consisting of 40,407 individuals of European descent, with additional genotyping in 7170 Europeans, yielded genome-wide significant associations of QRS duration with common variants in 22 loci. Variations in four of these loci (locus 1, *SCN5A/10A*; locus 2, *CDKN1A;* locus 8, *TBX5;* and locus 21, *DKK1*) were previously associated with QRS duration in smaller independent studies using both candidate gene and genome-wide approaches.7–9 The 22 loci include genes in a number of interconnected pathways, including some previously known to be involved in cardiac conduction, such as sodium channels, calcium-handling proteins, and transcription factors, as well as novel processes not known to be involved in cardiac electrophysiology, such as kinase inhibitors, growth factor-related genes, and others.

The electrocardiographic QRS interval reflects ventricular depolarization and conduction time. Ventricular myocyte depolarization occurs via cardiac membrane excitatory inward currents mediated by voltage-gated sodium channels.19 The primary determinants of conduction velocity are the magnitude of excitatory inward currents flowing through these

sodium channels, the extent of cell-to-cell communication via gap junction/connexin coupling, and cell and tissue architecture and morphology.19 Multiple pathways suggested in this study determine or modulate these key components of ventricular depolarization and conduction. Candidate genes in these pathways are briefly discussed in Box 1.

Box 1

Noteworthy genes within loci associated with QRS duration

Of the 22 loci identified, common variants in four loci (*SCN5A/SCN10A, CDKN1A, TBX5,* and *DKK1*) were previously associated with QRS duration in genetic association studies. Mutations in two (*SCN5A* and *TBX5*) lead to inherited syndromes associated with conduction disease. Animal experiments demonstrate a role for several additional loci (*HAND1, TBX3,* and *TBX5*) in cardiac ventricular conduction, as detailed below. The remainder are novel QRS loci, and their role in cardiac conduction remains to be elucidated.

1. **Cardiac sodium channel genes:**
    - ***SCN5A* (locus 1):** *SCN5A* encodes the cardiac $Na_v1.5$ sodium channel and is well known to influence cardiac conduction, as well as other cardiovascular and electrophysiologic phenotypes.20, 21
    - ***SCN10A* (locus 1):** *SCN10A* encodes the $Na_v1.8$ sodium channel, present in both ventricular myocardium and conduction fibers. Selective *SCN10A* blocker prolongs QRS interval.
2. **Calcium handling proteins:**
    - ***CASQ2* (locus 18):** *CASQ2* regulates opening of the ryanodine receptor (*RYR2*).37, 38 Cellular depolarization via Na-channels triggers calcium influx through L-type calcium channels, which in turn provokes *RYR2*-mediated calcium release from the sarcoplasmic reticulum. *CASQ2* mutations have been associated with catecholaminergic polymorphic ventricular tachycardia.39, 40
    - ***PLN* (locus 3):** Calcium uptake into the sarcoplasmic reticulum by SERCA2a is regulated by phospholamban (*PLN*).41 The phosphorylation state of *PLN* is dependent on signaling pathways involving phosphatases and kinases including *PRKCA*.41 We previously demonstrated that this locus is associated with both cardiac electrical properties (QT interval duration, heart rate) and size (left ventricular end diastolic dimension) in GWA analyses.11, 12, 42, 43
    - ***PRKCA* (locus 16):** Protein kinase C alpha activity affects dephosphorylation of the sarcoplasmic reticulum Ca^{2+} ATPase-2 (SERCA-2) pump inhibitory protein phospholamban (PLN), and alters sarcoplasmic reticulum Ca^{2+} loading and the Ca^{2+} transient.44
    - ***STRN* (locus 12):** Striatin is a Ca^{2+}/calmodulin binding protein that directly binds to caveolin scaffolding protein. Striatin has recently been implicated in a canine model of arrhythmogenic right ventricular cardiomyopathy.45, 46
3. **Transcription factors:**
    - ***TBX3* (locus 9) and *TBX5* (locus 8):** *TBX3* and *TBX5* encode transcription factors found in the cardiac conduction system. *TBX5*

(activator) competes with *TBX3* (repressor) for the regulation of working myocardial genes such as *GJA1*.47, 48 Common variations near *TBX3* and *TBX5* were associated with PR and QRS durations.9, 13 Mutations in *TBX3* and *TBX5* have been associated with rare inherited syndromes manifested by an array of defects including ventricular structural and/or conduction defects.

- ***TBX20* (locus 6):** *TBX20* demarcates the left and right ventricles49 and mutations i*n TBX20* have been implicated in multiple structural defects in mouse and human models.50, 51
- ***HAND1* (locus 5):** *HAND1* encodes a transcription factor essential to cardiac morphogenesis,52 with a mutation identified in human hearts with septal defects.53 Over-expression of Hand1 in the adult mouse heart leads to loss of connexin43 (*GJA1)* expression, QRS prolongation, and predisposition to ventricular arrhythmia.54
- ***NFIA* (locus 4) and *KLF12* (locus 19):** Little is known about the role of Nuclear Factor One (NFIA) and Kruppel like protein 12 (KLF12) in cardiac tissue development.

4. **Cyclin dependent kinase inhibitors:**
   - ***CDKN1A* (locus 2):** *CDKN1A* is a negative regulator of cell cycle entry into G2/M phase, and is upregulated by *ERBB2* activation. *ERBB2,* a member of the EGF receptor family of tyrosine kinases, is essential for proper heart development, and its ligand neuregulin-1 promotes formation of the murine cardiac conduction system.55 Furthermore, *ERBB2* can modulate gap junction assembly and alter appropriate phosphorylation of connexin 43 in glial cells.56 In addition, *CDKN1A* is upregulated by *PRKCA* (locus 16).57
   - ***CDKN2C* (locus 15):** A member of the family of cyclin-dependent kinase inhibitors that prevent the activation of the CDK kinases, thus functioning as a cell growth regulator that controls cell cycle G1 progression.
5. **Other pathways:**
   - ***CRIM1* (locus 14):** *CRIM1* (locus 14), a cell-surface transmembrane protein that may bind to various members of the TGF-beta superfamily of ligands, is expressed in mouse and human cardiac tissues.58, 59 *CRIM1* interacts with bone morphogenetic proteins, which induce the expression of *CDKN1A* (p21).58, 60
   - ***LRIG1* (locus 20):** *LRIG1* is upregulated in malignancies. It negatively regulates the proto-oncogenic, tyrosine kinase receptor family *ERBB2*.61
   - ***SETBP1* (locus 11):** *SETBP1* (locus 11) encodes a ubiquitously expressed protein that binds to the *SET* gene.62 The *SETBP1-SET* interaction has been hypothesized to be a component in tumor development.
   - ***TKT* (locus 13):** Transketolase (TKT) is a ubiquitous enzyme used in multiple metabolic pathways, including the pentose phosphate pathway.63

- *DKK1* (locus 21): *DKK1*, implicated in several tumors, inhibits the Wnt signaling pathway.64 Wnt signaling is an important modulator of connexin43 dependent intercellular coupling in the heart.65 In cardiac tissue it has an embryologic role with regard to axial development.66
- *SIPA1L1* (locus 7): *SIPA1L1* appears to play a role in non-canonical Wnt signaling and contributes to development.67

Our strongest association signal (locus 1) mapped in or near two voltage-gated sodium channel genes: *SCN5A* and *SCN10A. SCN5A* encodes the cardiac $Na_v1.5$ sodium channel and is well known for its role in cardiac conduction, and other cardiovascular and electrophysiologic phenotypes.20, 21 *SCN10A* encodes the $Na_v1.8$ sodium channel. We provide novel data demonstrating that the *SCN10A* transcript and product is preferentially expressed in the mouse His-Purkinje system compared with the ventricular myocardium, and that $Na_v1.8$ channel blockers result in QRS and HV interval prolongation, indicative of a slowing of impulse propagation in the specialized ventricular conduction system and delayed activation of the ventricular myocardium. Interestingly, Chambers et al. recently reported shortening of the PR interval in Scn10a knockout mice and concluded that Scn10a prolongs cardiac conduction and that rs6795970, encoding a $Na_v1.8$ A1073V variant, is a gain-of-function allele.8 Alternatively, the more rapid conduction they observed in the knockout mice could reflect compensatory upregulation of TTX-sensitive currents, a [22] phenomenon observed in $Na_v1.8$-deficient DRG neurons.

We, and others, demonstrated previously that, in addition to their association with QRS duration, variants in *SCN5A* and *SCN10A* are associated with atrial conduction (PR interval) and myocardial repolarization (QT interval), as well as atrial and ventricular fibrillation.8, 9, 13 These results emphasize the crucial role played by these genes in cardiac conduction and the generation of arrhythmias.

Calcium regulation is integral to impulse propagation, modulating cellular electrophysiology including sodium channel and gap junction function, as well as tissue architecture.20, 23, 24 Several of the loci associated with QRS duration contain genes directly related to calcium processes. As depicted in Supplementary Figure 4 and detailed in Box 1, these genes encode interrelated proteins that influence Ca^{2+} signaling (*PLN* in locus 3*; PRKCA* in locus 16; and *CASQ2* in locus 18) and downstream effects (*STRN* in locus 12).

Transcription factors regulating embryonic electrophysiologic development are critical for the integrity of impulse conduction.25 We identified six transcription factors (*TBX3* in locus 9; *TBX5* in locus 8; *TBX20* in locus 6; *HAND1* in locus 5; *NFIA* in locus 4; and *KLF12* in locus 19) in loci associated with QRS duration. Several of these transcription factors impact cardiac morphogenesis and may influence conduction by altering cellular and tissue architecture. Intriguingly, they may also have direct electrophysiologic consequences by modifying factors involved in impulse conduction. For example, *HAND1* and T-box factors regulate connexin 40 (*GJA5*) and/or connexin 43 (*GJA1*), and *TBX5* binds to the *ATP2A2 (SERCA2A)* promoter.26

Our study suggests a number of processes and pathways not previously known to be involved in cardiac electrophysiology, including cyclin dependent kinase inhibitors and genes related to tumorigenesis and cellular transformation. How these novel processes influence QRS duration remains to be defined.

In pleiotropic analyses, most variants influencing both PR and QRS, with the exception of *TBX3*, were concordant in effect direction, consistent with the known shared physiologic

processes underlying the two traits: depolarization and conduction time in the sino-atrial, atria and atrioventricular node (PR) and depolarization and conduction time in the ventricles (QRS). By contrast, although QRS (ventricular depolarization) and QT (ventricular repolarization) are moderately positively correlated, most loci influencing both traits showed discordant effect directions (with the exception of the *PLN* locus). Investigating the physiologic foundations for these concordant and discordant PR-QRS and QT-QRS relationships could be particularly informative for elucidating the mechanisms by which these loci influence cardiac depolarization, conduction and repolarization.

Several limitations of our study should be considered. First, although we have identified 22 loci significantly associated with QRS duration, the broad nature of linkage disequilibrium among common variants generally precludes an unambiguous identification of the culprit variant or of the functional gene. For several genes (*SCN5A*, *SCN10A*, *C6orf204*, *CASQ2*), there are common coding SNPs in high LD with the index SNP, which may lend some support for a functional role for these genes. Furthermore, our expression analysis in blood revealed very strong *cis*-eQTL associations for *TKT* and *CDKN1A*, lending additional support to these genes as functional candidates. It would be desirable to perform similar eQTL analyses based on expression data in myocardial cells or conduction tissue. For our top signal in *SCN10A*, a gene which until recently was not known to be expressed in the heart, our functional work in mice confirm that *SCN10A* is involved in ventricular depolarization and conduction. Further fine-mapping is needed at all 22 loci to conclusively test all genetic variation (rare and common) for a role in QRS modulation.

To minimize the potential for confounding due to population substructure, we limited the analyses to individuals of European descent, for whom we could assemble the largest number of samples. At the individual study level, the GWAS showed very little evidence for gross stratification (genomic inflation factor, $\lambda_{GC,}$ values ranged from 1.00 to 1.05). However, one of our QRS loci, mapping to *HAND1*/*SAP30L*, showed evidence of heterogeneity. In genetic association studies, heterogeneity can be due to sampling error, differences in phenotypic measurement, differences in LD structure between populations, technical artifacts, or genuine biological heterogeneity, but it would be difficult to conclude on the basis of our data here which is the most likely explanation.27

Our study underscores the power of a large genome-wide association study to extend prior biological understanding of cardiac ventricular conduction. Better understanding of the complex biologic pathways and molecular genetics associated with cardiac conduction and QRS duration may offer insight into the molecular basis underlying the pathogenesis of conduction abnormalities that can result in increased risk of sudden death, heart failure, and cardiac mortality.

Methods

Methods and any associated references are available in the online version of the paper.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Author contributions: Study concept and design:

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URL Section

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DAVID - http://david.abcc.ncifcrf.gov

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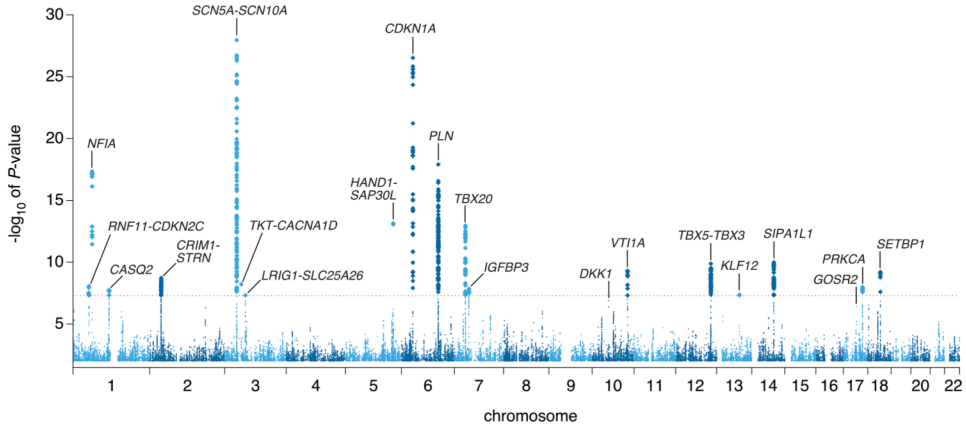

Figure 1. Manhattan plot

Manhattan plot showing the association of SNPs with QRS interval duration in a GWAS of 40,407 individuals. The dashed horizontal line marks the threshold for genome-wide significance ($P = 5 \times 10^{-8}$). Twenty loci (labeled) reached genome-wide significance. Two additional loci, *GOSR2* and *DKK1*, reached significance after genotyping of select SNPs in an additional sample of 7170 individuals (see **Results**).

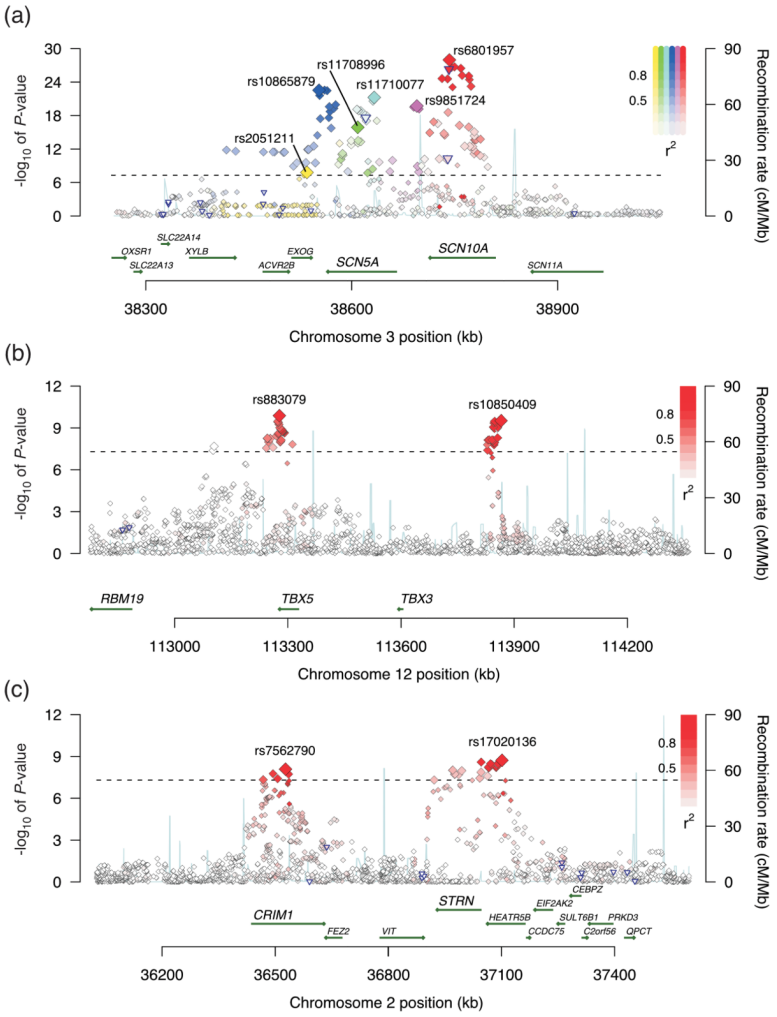

Figure 2. Association plots for select loci

Each SNP is plotted with respect to its chromosomal location (x-axis) and its *P*-value (y-axis on the left). The tall blue spikes indicate the recombination rate (y-axis on the right) at that region of the chromosome. The blue-outlined triangles indicate coding region SNPs. (a) Locus 1 (*SCN5A/SCN10A)* on chromosome 3: The six index signals are named with their rs numbers and highlighted in different colors (yellow, green, teal, blue, purple, and red). Other SNPs in linkage disequilibrium with the index SNP are denoted in the same color. Color saturation indicates the degree of correlation with the index SNP. (b) Locus 8 (*TBX5)* and locus 9 (*TBX3*) on chromosome 12. (c) Locus 12 (*HEATR5B/STRN*) and locus 14 (*CRIM1)* on chromosome 2.

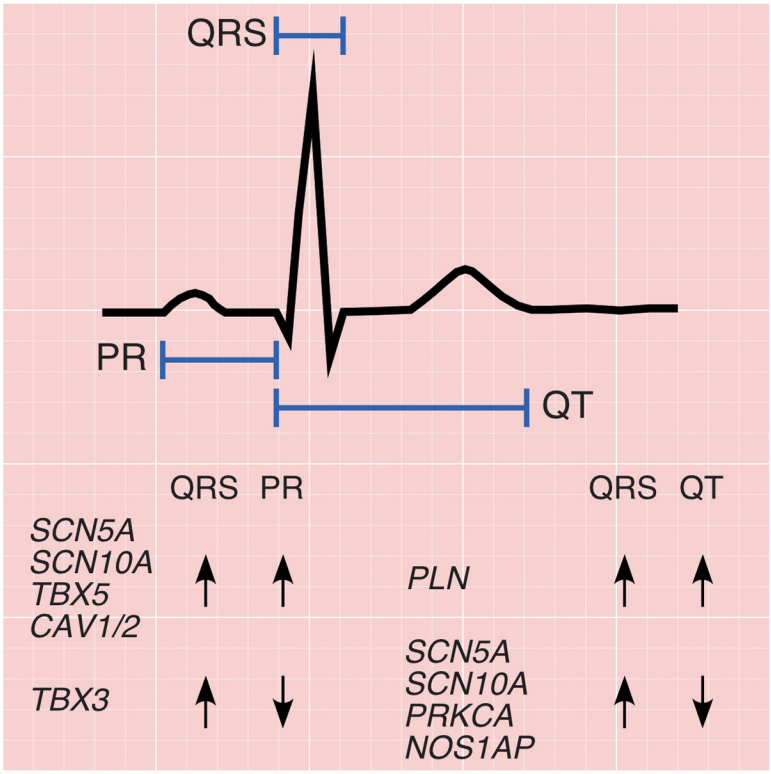

Figure 3. Pleiotropic associations of PR, QRS, and QT loci

Electrocardiographic tracing delineating the PR, QRS, and QT intervals. PR and QRS intervals reflect myocardial depolarization and conduction time through the atria and down the atrioventricular node (PR) and throughout the ventricle (QRS), and are weakly positively correlated (r=0.09). The majority of loci that influence both PR and QRS (*SCN5A, SCN10A, TBX5, CAV1/2)*, do so in a concordant fashion (i.e. variants that prolong PR also prolong QRS duration). The notable exception is a region on chromosome 12, where variants in the *TBX5* locus have a concordant effect whereas those in nearby *TBX3* have a discordant effect. By contrast, although QRS (ventricular depolarization) and QT (ventricular repolarization) are moderately positively correlated, the majority of loci (*SCN5A, SCN10A, PRKCA, NOS1AP)* that influence both phenotypes do so in a discordant fashion (i.e. variants that prolong QRS shorten the QT interval). The exception is the locus at *PLN*, where variants have a concordant effect.

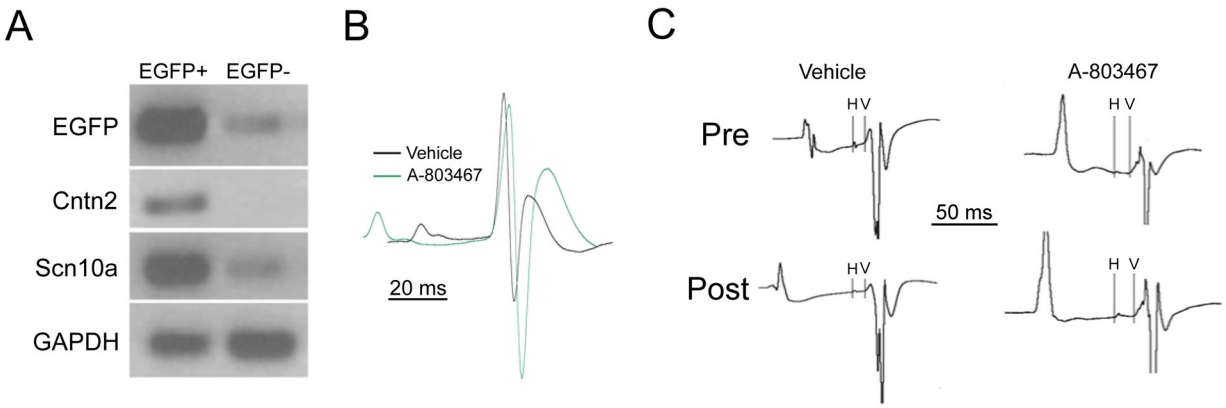

Figure 4. Expression and function of Scn10a in the murine heart

Panel A. Neonatal ventricular myocytes from *Cntn2-EGFP* BAC transgenic mice were FACS sorted and EGFP+ and EGFP- pools were analyzed by RT-PCR. Transcripts encoding *EGFP*, *Cntn2* and *Scn10a* were highly enriched in the EGFP+ fraction. Quantitative RT-PCR demonstrated 25.7 fold enrichment of *Scn10a*/$Na_v1.8$. *Panel B*. Representative telemetric electrocardiographic recordings (lead II configuration) obtained 30 minutes after administration of vehicle alone (black tracing) or the *Scn10a/Nav1.8* antagonist A-803467 (green tracing). The two tracings are aligned at the onset of the QRS wave and both PR interval and QRS interval prolongation were observed in drug-treated mice. *Panel C.* Representative intracardiac recordings showing HV intervals obtained prior to (Pre) and after (Post) administration of vehicle or A-803467. Significant HV prolongation was observed in drug-treated mice.

Table 1

Significant loci at $P<5\times10^{-08}$ in combined GWAS and candidate SNP meta-analysis

Locus	Chr	Index SNP	Coded/Non-coded Allele	AF	GWAS β	GWAS SE_{GC}	GWAS P_{GC}	I^2	Prevend β	Prevend *P*	$P_{Overall}$	Multi SNP β	Multi SNP *P*	Nearest Gene	SNP Annotation
1	3	rs6801957	**T**/C	0.41	0.77	0.07	1.10×10^{-28}	45.3	-	-	**1.10×10^{-28}**	0.54	**3.43×10^{-14}**	*SCN10A*	intron
	3	rs9851724	**C**/T	0.33	−0.66	0.07	1.91×10^{-20}	57.1	-	-	**1.91×10^{-20}**	−0.60	**5.78×10^{-16}**	*SCN10A/SCN5A*	intergenic
	3	rs10865879	**T**/C	0.41	0.77	0.07	1.10×10^{-28}	53.6	-	-	**1.10×10^{-28}**	0.33	1.67×10^{-04}	*SCN5A/EXOG*	intergenic
	3	rs11710077	**T**/A	0.21	−0.84	0.09	5.74×10^{-22}	23.8	-	-	**5.74×10^{-22}**	−0.44	**1.33×10^{-06}**	*SCN5A*	intron
	3	rs11708996	**C**/G	0.16	0.79	0.10	1.26×10^{-16}	0.0	-	-	**1.26×10^{-16}**	0.47	**7.23×10^{-06}**	*SCN5A*	intron
	3	rs2051211	**G**/A	0.26	−0.44	0.08	1.57×10^{-08}	0.0	-	-	**1.57×10^{-08}**	−0.18	3.71×10^{-02}	*EXOG*	intron
2	6	rs9470361	**A**/G	0.25	0.87	0.08	3.00×10^{-27}	14.6	-	-	**3.00×10^{-27}**	-	-	*CDKN1A*	intergenic
3	6	rs11153730	**C**/T	0.49	0.59	0.07	1.26×10^{-18}	5.3	-	-	**1.26×10^{-18}**	-	-	*C6orf204/SLC35F1/PLN/BRD7P3*	intergenic
4	1	rs9436640	**G**/T	0.46	−0.59	0.07	4.57×10^{-18}	51.2	-	-	**4.57×10^{-18}**	-	-	*NFIA*	intron
5	5	rs13165478	**A**/G	0.36	−0.55	0.07	7.36×10^{-14}	64.6	-	-	**7.36×10^{-14}**	-	-	*HAND1/SAP30L*	intergenic
6	7	rs1362212	**A**/G	0.18	0.69	0.09	1.12×10^{-13}	0.0	-	-	**1.12×10^{-13}**	-	-	*TBX20*	intergenic
7	14	rs11848785	**G**/A	0.27	−0.50	0.08	1.04×10^{-10}	0.0	-	-	**1.04×10^{-10}**	-	-	*SIPA1L1*	intron
8	12	rs883079	**C**/T	0.29	0.49	0.08	1.33×10^{-10}	8.3	-	-	**1.33×10^{-10}**	-	-	*TBX5*	3′-UTR
9	12	rs10850409	**A**/G	0.27	−0.49	0.08	3.06×10^{-10}	0.0	-	-	**3.06×10^{-10}**	-	-	*TBX3*	intergenic
10	10	rs7342028	**T**/G	0.27	0.48	0.08	4.95×10^{-10}	0.0	-	-	**4.95×10^{-10}**	-	-	*VTI1A*	intron
11	18	rs991014	**T**/C	0.42	0.42	0.07	6.20×10^{-10}	0.0	-	-	**6.20×10^{-10}**	-	-	*SETBP1*	intron
12	2	rs17020136	**C**/T	0.21	0.51	0.08	1.90×10^{-9}	0.0	-	-	**1.90×10^{-9}**	-	-	*HEATR5B/STRN*	intron
13	3	rs4687718	**A**/G	0.14	−0.63	0.11	6.25×10^{-9}	0.0	-	-	**6.25×10^{-9}**	-	-	*TKT/PRKCD/CACNA1D*	intron
14	2	rs7562790	**G**/T	0.40	0.39	0.07	8.22×10^{-9}	0.0	-	-	**8.22×10^{-9}**	-	-	*CRIM1*	intron
15	1	rs17391905	**G**/T	0.05	−1.35	0.23	8.72×10^{-9}	4.0	-1.17	0.005	**3.26×10^{-10}**	-	-	*C1orf185/RNF11/CDKN2C/FAF1*	intergenic
16	17	rs9912468	**G**/C	0.43	0.39	0.07	1.06×10^{-8}	28.2	-	-	**1.06×10^{-8}**	-	-	*PRKCA*	intron
17	7	rs7784776	**G**/A	0.43	0.39	0.07	1.42×10^{-8}	0.0	0.36	0.015	**1.28×10^{-9}**	-	-	*IGFBP3*	intergenic
18	1	rs4074536	**C**/T	0.29	−0.42	0.07	2.36×10^{-8}	0.5	-	-	**2.36×10^{-8}**	-	-	*CASQ2*	missense
19	13	rs1886512	**A**/T	0.37	−0.40	0.07	4.31×10^{-8}	0.0	-0.28	0.047	**1.27×10^{-8}**	-	-	*KLF12*	intron
20	3	rs2242285	**A**/G	0.42	0.37	0.07	4.79×10^{-8}	35.4	0.29	0.040	**1.09×10^{-8}**	-	-	*LRIG1/SLC25A26*	intron

Locus	Chr	Index SNP	Coded/Non-coded Allele	AF	GWAS β	GWAS SE_{GC}	GWAS P_{GC}	I^2	Prevend β	Prevend *P*	$P_{Overall}$	Multi SNP β	Multi SNP *P*	Nearest Gene	SNP Annotation
21	10	rs1733724	**A**/G	0.25	0.49	0.09	1.26×10^{-7}	0.0	0.34	0.035	$\mathbf{3.05\times10^{-8}}$	-	-	*DKK1*	intergenic
22	17	rs17608766	**C**/T	0.16	0.53	0.10	3.71×10^{-7}	13.8	0.92	4.7×10^{-5}	$\mathbf{4.75\times10^{-10}}$	-	-	*GOSR2*	intron, 3′

In each locus at least one marker exceeds the genome-wide significance threshold of $P < 5 \times 10^{-8}$. At locus 1, six signals were identified ($r^2<0.05$) that exceeded genome-wide threshold. In a multiSNP model that included all 6 SNPs, there was evidence that at least 4 of these SNPs were independently associated with QRS duration. The **bolded** allele is the coded allele. Beta values (β) estimate the difference in QRS interval in milliseconds per copy of the coded allele, adjusted for the covariates in the model. Chr, chromosome; AF, coded allele frequency; SE, standard error; GC, genomic control adjusted; UTR, untranslated region. AF is an average weighted by study size.